Reference: *Biol. Bull.* **182**: 305–323. (June, 1992)

Studies on the Structure and Function of the Larval Kidney Complex of Prosobranch Gastropods

BRIAN R. RIVEST

Department of Biological Sciences, State University of New York at Cortland, Cortland, New York 13045

Abstract. The larval kidneys of prosobranch gastropods have long been assumed to be involved in handling wastes, but with little supporting evidence. In this study, the larval kidneys of *Searlesia dira* and *Nucella canaliculata* were studied with light, electron, and fluorescence microscopy. They consist of three cell types: (1) a large external absorptive cell swollen with heterophagosomes and possessing an endocytotically active external surface; (2) an internal crystal cell with numerous vacuole-bound crystals of a calcium salt and with morphologically complex canaliculi; (3) an internal pore cell characterized by slit-pores that lead to subsurface cisternae, a tubular network, and one or two ciliated ducts that open into the hemocoel. Empirical evidence indicates that the absorptive cell rapidly takes up and stores albumen proteins from the capsular fluid. Absorptive cells were found in 17 of 19 species tested, representing three prosobranch orders, but were not found in 2 opisthobranch or 1 pulmonate species. We hypothesize that the absorptive cells have become specialized for the uptake of capsular albumen prior to the functional differentiation of the gut. However, the nutritional importance of the absorbed albumen proteins and the functions of the crystal and pore cells are presently unknown. No evidence for an excretory function was found for the larval kidney complex; it may be a vestigial protonephridium, the components of which have become disorganized and functionally altered.

Introduction

Larval kidneys are prominent structures found in the neck region in many prosobranch embryos. They have long been considered to excrete actively or to accumulate waste products (Bobretzky, 1877; Heymons, 1893; Glaser, 1904; Pelseneer, 1911; Franc, 1940). The hypothesis that these structures are organs of accumulation is attractive because it explains the large cells as an adaptation for encapsulated development (Pelseneer, 1911; Eisawy and Sorial, 1974). Embryos might better sequester their wastes than pollute the surrounding capsular fluid.

Prosobranch larval kidneys have been described as paired, laterally located uni- or multicellular structures that protrude from the region behind the velum, and are usually considered to be of ectodermal origin (Casteel, 1904; Portmann, 1930; Franc, 1941). Bobretzky (1877), Conklin (1897), Glaser (1904), and D'Asaro (1966) described them as enlarged ectodermal cells that either released excretory granules or were completely cast off before hatching. In *Ocenebra aciculata*, Franc (1940) found the larval kidneys to consist of a larger outer vacuolated cell overlying a smaller internal cell containing green vacuoles. He tried unsuccessfully to demonstrate experimentally an excretory function of the larval kidneys. However, he did find what he described as yolk platelets in the larger external cell of *O. aciculata* and *Thais haemastoma* (Franc, 1940, 1941). Although Franc realized that the presence of yolk would suggest that larval kidneys were involved in something other than excretion, he retained the classical assumption that they were organs of waste accumulation and hypothesized that the yolk supplied the energy used for waste acquisition (Franc, 1941).

Although the presence of larval kidneys in prosobranch embryos has been reported by numerous authors, they are poorly understood. The material accumulated in the enlarged ectodermal cells of the larval kidneys has not been identified, although Glaser (1904) claimed that an aqueous extraction of larval kidney cells contained dilute urea. Furthermore, little is known of the ultrastructure of prosobranch larval kidneys. In this study I describe the ultrastructure of the three cells that make up the larval

Received 30 December 1991; accepted 17 March 1992.

kidney in the marine snail *Searlesia dira* and the ontogeny of these cells, from the early trochophore to the mid-veliger stage. Similar observations of the larval kidneys of *Nucella canaliculata* are included. Morphological and experimental evidence shows that the large size of the larval kidneys is due to endocytotically absorbed albumen proteins, not stored waste products. Twenty other species of gastropods were tested for larval kidneys that absorb dissolved proteins from the surrounding fluid.

Materials and Methods

Collection of egg masses and egg capsules

Most egg masses or capsules used in this study were collected in intertidal or subtidal areas around San Juan Island, Washington, or were obtained from animals maintained in aquaria at the Friday Harbor Laboratories. Egg capsules of *Ocenebra japonica* were collected on oyster flats in southern Puget Sound, Washington, and those of *Nucella lapillus* at York Beach, Maine.

Specimen preparation for light and electron microscopy

Searlesia dira and *Nucella canaliculata* embryos to be examined with scanning electron microscopy were removed from their capsules, rinsed in filtered seawater, relaxed with 7.5% magnesium chloride and fixed for one to two hours at room temperature in 2% osmium tetroxide in 1.25% sodium bicarbonate at pH 7.2 (Wood and Luft, 1965). The embryos were then rinsed in distilled water, dehydrated in ethanol, critical point dried, mounted, and coated with gold. The embryos were examined with an ETEC Autoscan or a JEOL JSM-35 scanning electron microscope.

Satisfactory fixation of larval kidney cells for transmission electron microscopy was difficult. Fixatives that appeared suitable for adjacent ectoderm and subjacent endoderm often produced fixation artifacts in the larval kidney, particularly in the outer absorptive cell. The heterophagosomes of this voluminous cell appeared particularly sensitive to the osmolarity of the fixative. The larval kidneys of *Searlesia dira* were most satisfactorily fixed in a cacodylate buffered glutaraldehyde solution containing ruthenium red, a method modified from Cavey and Cloney (1972). Embryos removed from their capsules and rinsed with seawater were placed in 2% glutaraldehyde in a solution buffered by 0.2 *M* sodium cacodylate adjusted to 1000–1100 mOsM with sucrose and containing 0.05% ruthenium red and 0.002 *M* calcium chloride. Final pH was adjusted to 7.4. This fixative did not work well for the larval kidneys of *Nucella canaliculata*, which showed better preservation using a 3% glutaraldehyde solution containing 0.1 *M* sodium cacodylate and 0.001 *M* calcium chloride and adjusted to 1000–1100 mOsM with sodium chloride. This worked satisfactorily for light microscopy but produced severe artifacts at the ultrastructural level.

The embryos were postfixed in 2% osmium tetroxide in freshly mixed 1.25% sodium bicarbonate (pH 7.2) for one hour at room temperature. The embryos were then dehydrated and embedded in EPON (Luft, 1961).

One micrometer thick sections for light microscopy were stained with a mixture of Azure II and methylene blue in 0.5% sodium borate (Richardson *et al.*, 1960). Serial thin sections for transmission electron microscopy were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Philips EM-300 electron microscope.

The *Searlesia dira* developmental stages that were sectioned included early trochophores prior to or just after the initiation of nurse egg feeding, late trochophores near or just after the end of nurse egg feeding, early veligers with the cephalopedal elements elevated from the main embryonic body and the mantle fold near the anterior end, and mid-veligers with greater differentiation of the cephalopedal elements and a small mantle cavity. The *Nucella canaliculata* developmental states that were sectioned included late trochophores, early veligers, and mid-veligers.

Exposure of embryos to test solutions

To study the uptake of material by the larval kidneys, embryos were initially exposed to isosmotic solutions of ferritin. Later experiments included placing embryos in isosmotic solutions containing fluorescein, bovine serum albumin (BSA), capsular albumen, fluoresceinisothiocyanate labelled BSA (FITC-BSA, Sigma Chemical Company), and/or FITC-capsular albumen.

The capsular albumen proteins of *Searlesia dira* were labelled with FITC as follows: (1) seventy capsules less than three weeks old (before the embryos had begun to feed on the nurse eggs) were opened in 4°C filtered seawater containing 10 μg/ml of the antibiotic rifampicin. The albumen was separated from the embryos and nurse eggs by using a 152 μm Nitex screen. (2) The seawater-diluted albumen was centrifuged to remove any debris. (3) The supernatant was placed in dialysis tubing on a bed of Aquacide (CalBiochem) to extract water and concentrate the proteins. (4) The protein content of the solution was estimated by spectrophotometrically measuring absorbance at 280 mμ and assuming the extinction coefficient of capsular albumen is 6.2 (intermediate between the extinction coefficients of BSA and human serum albumin). (5) Sufficient $NaHCO_3$ was added to make a 0.1 *M* solution with a pH of about 9.2. (6) FITC (approximately one-tenth the weight of the estimated protein in the solution) was dissolved in one drop of 0.5 *N* NaOH, then the buffered albumen solution was added. (7) The

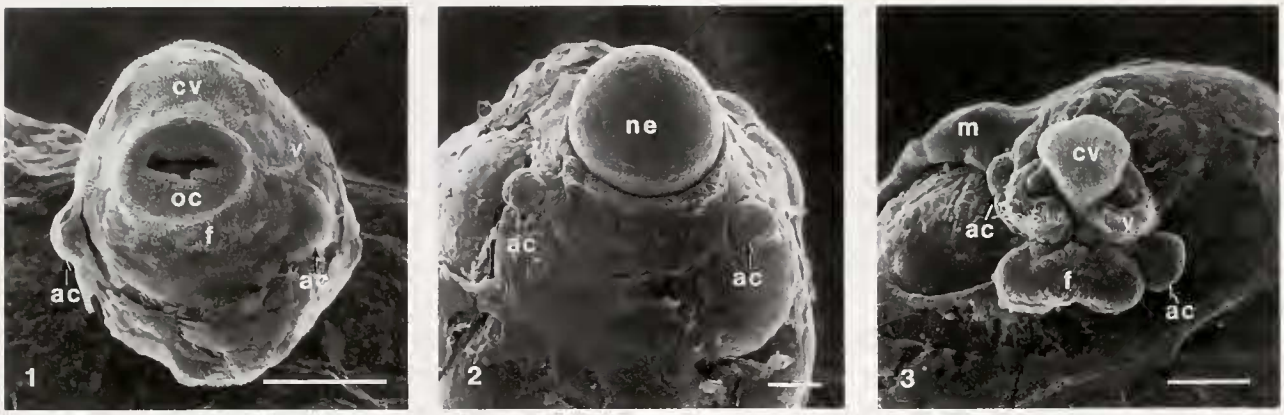


Figure 1. SEM of a *Searlesia dira* embryo shortly after the onset of feeding on nurse eggs showing the protruding absorptive cells of the larval kidneys.

Figure 2. SEM of an older feeding stage of *S. dira* fixed while swallowing a nurse egg. The absorptive cells are larger and protrude further from the embryo's surface.

Figure 3. SEM of an *S. dira* intracapsular veliger showing the developing cephalopedal structures and the bulbous nature of the left absorptive cell.

Scale bars represent 100 μm; ac, absorptive cell of the larval kidney; cv, cephalic vesicle; f, foot; oc, oral cilia; m, mantle margin; ne, nurse egg; v, velum.

mixed solutions were incubated at 20°C for 20 h. (8) Unreacted FITC was removed by adding 50 mg powdered charcoal per mg FITC, spinning the charcoal down after 30 min and repeating this step. (9) The pH was adjusted to that of seawater with 0.1 *N* HCl and the final solution was dialyzed against seawater for four hours. This solution contained approximately 0.37 mg/ml protein, as determined spectrophotometrically.

Embryos were transferred to a test solution at 12°C after first being removed from their capsules and rinsed in filtered seawater. After periods of 10 min to 4 h, the embryos were rinsed again in seawater and examined using light, fluorescence, or transmission electron microscopy.

To examine the nature of the crystals found in one of the inner cells of *Searlesia dira* larval kidneys, live and heat-killed embryos were examined with polarized light while exposed to one of the following solutions: Millonig's phosphate buffer at pH 3.5 to 7.5; 0.1 *M* glycine at pH 9.2; buffered (pH 8.5) and unbuffered (pH 3.0) 10% formalin in seawater; 0.1 *M* EGTA (pH 9.0) in seawater; 100% ethanol; 10% Triton-X 100 in distilled water; saturated aqueous calcium chloride. Some live embryos were placed in a 2% alizarin red S solution in seawater for 48 h.

Some *Searlesia dira* embryos at the early veliger stage of development were micro-injected in the hemocoel behind the velum with an isosmotic ferritin solution. After 10 to 60 min, these embryos were fixed and prepared for examination with transmission electron microscopy.

Larval kidney histochemistry

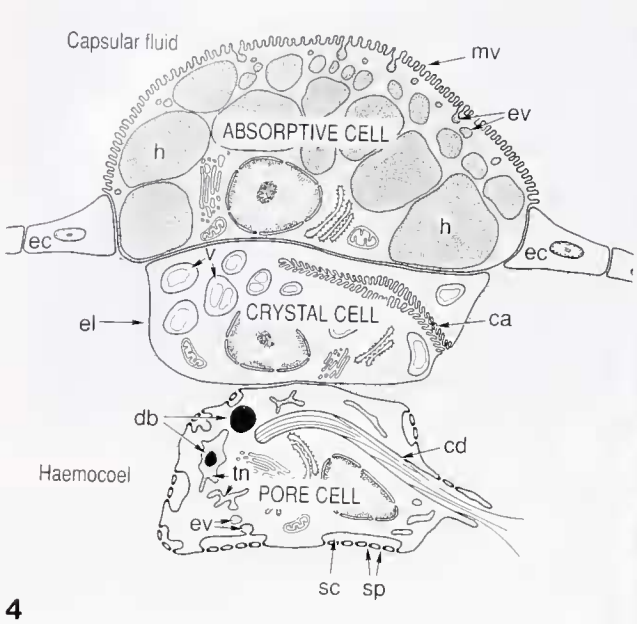
The periodic acid-Schiff reaction was performed on 10 μm thick serial paraffin sections of *Searlesia dira* embryos fixed in Carnoy's fixative (Humason, 1972). As a control for glycogen, some sections were incubated in saliva at 37°C for 1 h before applying the PAS technique. The hexamine silver method (Pearse, 1972) was used to test for urates in paraffin sections of embryos fixed in 100% ethanol or Carnoy's fixative.

Results

Searlesia dira

External morphology of the larval kidneys. The two larval kidneys develop laterally on the embryo just posterior to the mouth (Fig. 1). They are discernible as protrusions on live embryos within four weeks of oviposition at 12°C, before the embryos have begun to feed on nurse eggs. At this early trochophore stage, the larval kidneys are circular in outline and 40 to 50 μm in diameter. During the one to two and a half weeks that the embryos are feeding on nurse eggs and for a week or two afterwards, the larval kidneys enlarge, bulging outward from the embryo (Fig. 2). As they enlarge they become more hemispherical, but their shape and size varies among embryos.

After nurse egg feeding has ended, the cephalopedal elements grow out from the previously roundish embryo. At this point the larval kidneys are on the neck region of these veligers, posterior to the developing velar lobes but anterior to the forming mantle cavity. The right larval kidney has typically become elongate in the dorso-ventral axis, extending dorsally from the midlateral line. In contrast, the left larval kidney has become more bulbous, protruding at the level of, or slightly ventral to, the left lateral midline (Fig. 3).



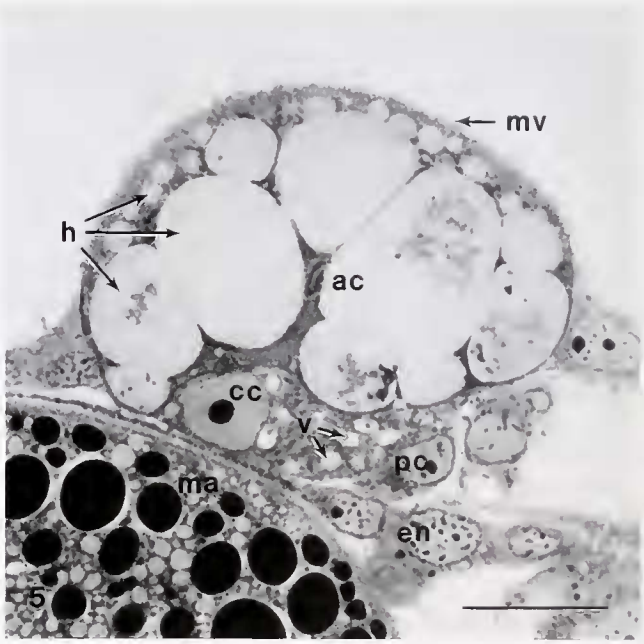


Figure 4. Schematic drawing of a section through the three cells making up a larval kidney complex in a *Searlesia dira* veliger. Proportions are not relative. The crystals in the vacuoles of the crystal cell dissolve during fixation and were not seen in sections.

Figure 5. One micrometer thick section of the larval kidney complex in *S. dira*. The pore cell can only be positively identified in fixed material with TEM.

Scale bar represents 25 μm; ac, absorptive cell; ca, canaliculus; cc, crystal cell; cd, ciliated duct (one of two); db, dark body; ec, ectoderm; el, external lamina; en, endoderm; ev, endocytotic invaginations and vesicles; h, heterophagosomes; ma, macromere of swallowed nurse egg; mv, microvilli; pc, pore cell; tn, tubular network; sc, subsurface cisternae; sp, split pores; v, vacuoles.

The larval kidneys have achieved their maximum size by the time the advancing mantle margin begins to form the mantle cavity. The elongate right larval kidney can be up to 100 μm long and 35 μm wide, while the more spherical left larval kidney may reach 80 μm in diameter. The larval kidneys have no distinctive coloration, but appear more yellowish-white than adjacent ectodermal cells. Both larval kidneys maintain their relative shape, position, and size during the intracapsular veliger phase of development. During the intracapsular metamorphosis that follows, the larval kidneys diminish in size until they are no longer discernible before the end of velar lobe resorption. There is no evidence suggesting that the larval kidneys fall off or spontaneously release their contents into the lumen of the capsule. There is also no evidence of a pore near the larval kidney as reported in *Ilyanassa obsoleta* (Tomlinson, 1987).

Ultrastructure of the larval kidneys of Searlesia dira. Each larval kidney is a complex of three cells: absorptive, crystal, and pore cells. An exception was found in one sectioned embryo that had a small second crystal cell adjacent to the main one. Absorptive cells are named after their ability to absorb external proteins. Crystal cells have been reported in prosobranch larval kidneys by Portmann (1930) and Fioroni (1985). Larval kidney pore cells have surface specializations similar to pore cells found in molluscan connective tissue (Sminia and Boer, 1973), although they are called rhogocytes by Fioroni *et al.* (1984). Each cell type is identifiable in thin sections of the prefeeding trochophores, the earliest stage examined. Figure 4 schematically illustrates the distinctive features of these three cells in an early veliger.

Absorptive cell. The absorptive cell is the outermost and largest of the three cells that comprise the larval kidney complex (Figs. 4, 5). The external surface of the absorptive cell is exposed to the capsular fluid surrounding the embryo. Morphological and experimental evidence indicate that it is a surface active in receptor-mediated endocytosis. It is elaborated into numerous microvilli up to 1.8 μm long that have endocytotic vesicles forming around their bases (Fig. 6). Adjacent endocytotic vesicles apparently fuse shortly after their formation, and the resulting small heterophagosomes fuse with larger, deeper heterophagosomes. The size of the absorptive cell may be due mostly to a few large heterophagosomes, which can reach 35 μm in diameter. In live embryos, large heterophagosomes can easily be detected in the absorptive cell with light microscopy; they appear refractile and colorless.

When embryos are soaked in a ferritin solution for 10 min or more, ferritin is found in endocytotic vesicles and heterophagosomes (Fig. 6). Thus, the heterophagosomes contain material brought into the cell by endocytosis. In untreated embryos, the contents of the heterophagosomes appear fairly uniform with scattered granules, fibrils, or membranous structures. The density of the contents varies among the heterophagosomes within a cell. This appears to be due to differences in concentration of the contents, not to a segregation of material brought into the cell. This is supported by the fact that ferritin is eventually found in all the heterophagosomes in treated embryos. After an embryo has soaked 20 min in a reddish ferritin solution at 15–18°C, the absorptive cell takes on a light reddish color. This color is restricted to small heterophagosomes in the external cortex of the cell. After another 30 min in the solution, the color can be perceived in larger heterophagosomes deeper inside the cell. After an hour or more, the absorptive cells are deep red. This contrasts with the rest of the ectoderm which has remained translucent and colorless. There is also no concentration of ferritin in cells lining the gut.

The heterophagosomes comprise an estimated 80–90% of the absorptive cell's volume when the cell has reached its maximum size. The nucleus is usually centrally located, surrounded by heterophagosomes that indent the nuclear membrane. Few organelles occur in the apical cytoplasm; Golgi cisternae, mitochondria, and dense concentrations of rough ER are found laterally and basally. The ground cytoplasm appears more electron-dense than that of adjacent ectodermal cells or the subjacent crystal cell (Fig. 7, upper right).

The heterophagosomes showed a positive periodic-acid Schiff reaction which did not change after incubation of sections with saliva. This indicates the presence of a carbohydrate or carbohydrate-protein complex, but not of glycogen (Humason, 1972). Also, glycogen was not seen in electron micrographs. Tests for urates were negative, and no crystals or large granules were found in the heterophagosomes or cytoplasm. The heterophagosomes contained a few myelinic bodies, but these were uncommon and probably fixation artifacts. Experimental evidence (described below) indicates that the heterophagosomes contain albumen proteins taken in from the capsular fluid.

Crystal cell. The crystal cell is subjacent to the absorptive cell (Figs. 4, 5, 7). The plasmalemmae of these two cells are closely apposed, although occasionally small intercellular spaces are found. Junctional complexes between them were not found. The crystal cell covers most of the inner surface of the absorptive cell.

In live embryos, the crystal cell can be discerned under the absorptive cell because of its crystals. These crystals can be seen most easily in early veliger stages. They are irregularly shaped and usually occur singly within vacuoles, but may be in clusters of up to five crystals. The crystals measure up to 5 μm in diameter and are birefringent under polarized light. There are from 20 to 60 crystals in each crystal cell in early veligers. The crystals are solubilized, as determined by the loss of birefringence, within 2–5 min in heat killed embryos in pH 3.5 Millonig's phosphate buffer or in pH 9.0 0.1 *M* EGTA in seawater. They quickly dissolved when live embryos were fixed in unbuffered pH 3.0 10% formalin, but not in buffered pH 8.5 formalin. The crystals were not solubilized within 10 min in phosphate buffers with a pH from 5.0 to 7.5, in pH 9.2 0.1 *M* glycine, in ethanol, or in live embryos in the EGTA solution. The crystals quickly dissolved in 1 *N* NaOH and in a 10% solution of the detergent Triton-X 100 as soon as the vacuoles containing the crystals broke open. However, in 10% Triton-X 100 made up in a saturated calcium chloride solution, the crystals dissolved more slowly after the vacuoles were lysed. In live embryos that were soaked in a filtered 2% alizarin red S solution in seawater for 24 h, the normally lightly greenish-yellow crystals were reddish, as was the growing edge of the shell.

The distinctive features of the crystal cell in sectioned material are the presence of numerous membrane-bound vacuoles and a complex canaliculus. The vacuoles are few and small in pre-feeding trochophores (Fig. 8), but they become larger and more numerous as development proceeds (Fig. 7). The vacuoles originally contained the crystals that were solubilized during fixation. The appearance of the vacuolar contents varies with the fixation used, but they generally appear light under light and electron microscopy. In material fixed in cacodylate buffered glutaraldehyde with ruthenium red and post-fixed in osmium tetroxide, the contents include small granules, fibrils, and membranous or myelinic bodies. Larger electron-dense granules are occasionally found, especially when osmium tetroxide is used as the primary fixative. These may be the remnants of partially dissolved crystals.

The canaliculus that also characterizes the crystal cell is morphologically complex and ends blindly. Its development is associated with the development of a ciliated duct in the pore cell. In the early trochophore, the opening of the canaliculus faces the pore cell in an area surrounded by a zonula adherens between these two cells (Fig. 8). At this stage there is no communication between the lumen of the canaliculus and the nearby hemocoel. The walls of the canaliculus possess numerous plications and microvilli that protrude into the lumen, vastly increasing the surface area of the canaliculus. Cilia arising from a duct in the pore cell extend into the lumen of the canaliculus. The canaliculus can extend for most of the length of the crystal cell before it ends. It has many short side branches, and it may bifurcate near its blind end. As development proceeds, the plications and microvilli become more nu-

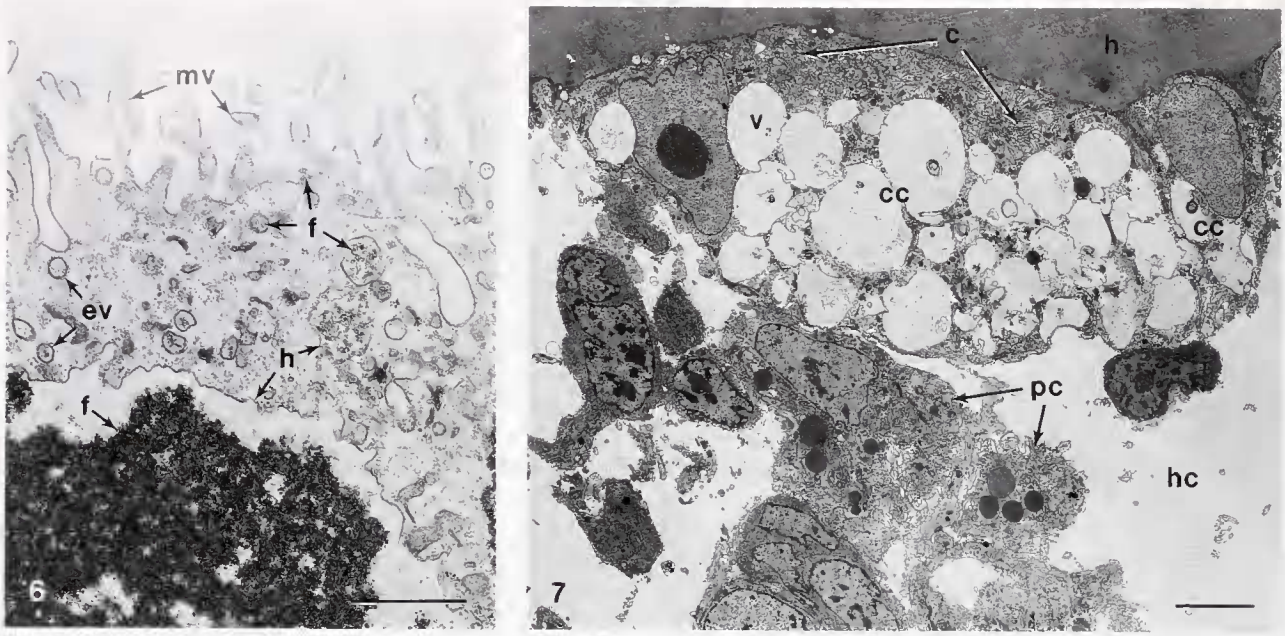


Figure 6. TEM of the external border of an absorptive cell in a *Searlesia dira* trochophore that had been soaked in a ferritin solution for 2 h prior to fixation, illustrating the endocytotic uptake of ferritin and its accumulation in heterophagosomes. Scale bar represents 1 μm.

Figure 7. TEM showing two crystal cells, a pore cell and the inner edge of the absorptive cell in a larval kidney of an early *S. dira* veliger. Most larval kidney complexes have only one crystal cell. The pore cell at this stage has little contact with the crystal cell, and has become branched. Scale bar represents 5 μm.

c indicates the canaliculus; cc, crystal cell; ev, endocytotic vesicles; f, ferritin; h, heterophagosomes of absorptive cell; hc, hemocoel; mv, microvilli; pc, pore cell; v, vacuole.

merous and more cilia grow into the canaliculus from the pore cell as the canaliculus becomes longer (Fig. 9). Occasional invaginations of coated membrane in the canalicular wall and nearby coated vesicles suggest receptor-mediated endocytotic uptake of material from the canalicular lumen.

By the time the embryo has developed into an early veliger, the crystal cell has lost its close association with the pore cell. These two cells still adjoin each other in a small area, but junctional complexes between them are no longer found. With the separation of these cells, the cilia from the pore cell no longer extend into the canaliculus of the crystal cell. Both the canaliculus of the crystal cell and ciliated duct of the pore cell now open into the hemocoel. However, the lumen of the canaliculus has become generally occluded by plications and microvilli. These structures fill the canalicular opening so that it is reduced to a web of anastomosing slits (Fig. 10). Only at one or two sites inside the cell does the lumen of the canaliculus open up into spaces that are lined by microvilli (Fig. 11). At these locations, the contents of the lumen appear finely granular. Elsewhere there is very little space within the walls of the canaliculus. Even the finger-like side branches contain long microvilli that fill the lumen. A transverse section of these filled branches appears as two concentric rings of unit membrane (Fig. 11).

Pore cell. The pore cell is the inner most of the three cells which comprise the larval kidney complex. It is characterized by slit-pores on the cell surface leading to subsurface cisternae, an extensive network of membranous tubules, and one or two ciliated ducts that eventually open into the hemocoel.

In the prefeeding trochophore, the pore cell is discoid and adheres closely to the hemocoelic side of the much larger crystal cell (Fig. 8). It is relatively undifferentiated, having few if any slit-pores and little tubular network. At this stage about six cilia arising from a shallow invagination have grown into the canaliculus of the crystal cell. The invagination deepens and the cilia become more numerous as the cell continues to differentiate (Fig. 9). As the crystal cell and pore cell become less closely associated, the cilia may extend into the hemocoel.

As the pore cell enlarges and differentiates, it grows away from the crystal cell into the hemocoel and becomes branched. As seen by Nomarski differential interference contrast microscopy, the branches attenuate into filopodia that extend toward the esophagus or to nearby ectodermal

cells. The cell body is thus suspended in the hemocoel underneath the crystal cell.

The cell surface develops slightly flattened, interdigitating finger-like processes that lie over spaces called subsurface cisternae (Skelding and Newell, 1975) (Figs. 12–14). The subsurface cisternae are typically around 200 to 300 nm deep, but their width varies considerably and in relation to the number of processes that form their roof. Although subsurface cisternae eventually develop under most of the cell surface, they are not interconnected.

The interdigitations are separated by slit-pores of a uniform 22.5 to 24.0 nm width that connect the subsurface cisternae with the hemocoel. The gap may contain fibrous material, but there was no evidence suggesting that the slit-pore is spanned by a diaphragm or membrane. An external lamina covers the surface of the pore cell, although in many preparations it has separated from the cell surface, possibly a result of fixation (Fig. 14). A dense material lies beneath the plasmalemma on either side of the slit-pores (Figs. 12, 13).

The floor of the subsurface cisternae consists of an endocytotically active plasmalemma. Invaginations of coated membrane pinch off to form vesicles that contain material brought in through the slit-pores (Fig. 14). The slit-pores could be a site of filtration, although particles as large as ferritin injected into the hemocoel pass through the slit-pores into the subsurface cisternae and are taken up by endocytotic vesicles (Fig. 15). The external lamina, however, may act as a partial barrier to the passage of ferritin because the density of ferritin within the subsurface cisternae was less than that in the hemocoel.

An extensive tubular network is found throughout the cytoplasm of the pore cell. This anastomosing network contains material taken in from the subsurface cisternae by endocytosis. Ferritin injected into the hemocoel is found within 10 to 15 min in endocytotic vesicles and the tubular network (Fig. 15). In non-injected embryos, a very fine fibrillar material is found adjacent to coated membranes in the subsurface cisternae, in endocytotic vesicles, and in some parts of the tubular network (Fig. 16).

Some inflated parts of the tubular network contain dark bodies. The dark bodies appear homogeneous and consist of a finely granular material. Small dark bodies may be surrounded by regions of the lumen of the tubular network (Fig. 16, near top), but this is not true with large dark bodies (Figs. 7, 15, 16, near bottom, and 17). Dark bodies are apparently formed by a condensation of material taken up endocytotically from the subsurface cisternae and channeled into the cell by the tubular network. Although the dark bodies appear electron dense in the transmission electron microscope, they are colorless and refractile in live embryos.

Golgi complexes are often found near enlarged regions of the tubular network (Fig. 17). Small vesicles about 80 nm in diameter frequently found near the Golgi cisternae may arise from the ends of the Golgi cisternae and fuse with the tubular network.

During development, the ciliated inpocketing of the pore cell deepens until the cilia arising from the proximal end of the duct no longer extend distally beyond the duct opening into the hemocoel. A second ciliated duct develops, apparently separate from any association with a canaliculus in the crystal cell. Regardless, it is morphologically indistinguishable from the first duct. As development of these ducts proceeds, additional cilia form, both at the proximal ends and scattered along the walls. Transverse sections of each duct reveal from 10 to 70 cilia. The number becomes greater in distal regions of the ducts and with greater cell differentiation. Occasional inpocketings of coated membrane indicate a low level of endocytotic activity. The duct walls are surrounded by a continuous plasmalemma for most of their length, but are perforated by a few slit-pores in a some distal areas (Fig. 16). In contrast to the other slit-pores, these are openings between the duct lumen and the hemocoel. When present, there are usually only one to five slit-pores in a transverse section through a ciliated duct. Even when they are more numerous, they have never been seen to occur around more than one third of the periphery of the duct, nor do they occur along much of the duct length. Presumably, the beating of the cilia in the duct could draw hemolymph in through the slit-pores. However, no ferritin was found in the ciliated ducts of embryos that had been injected with ferritin fifteen minutes before fixation.

The internal walls of the duct occasionally possess slit-pores leading to subsurface cisternae (Fig. 16). These structures have the same morphology as those on the peripheral cell surface and show some endocytotic activity, but are not common.

In the mid-veliger stage, prior to the formation of a mantle cavity into which the cephalopedal elements can be withdrawn, the distal region of each ciliated duct is found in a tubular outgrowth from the pore cell. The walls of this tube may be as thin as 0.3 μm, but are only occasionally perforated by slit-pores even though some slit-pores leading to subsurface cisternae are found on the hemocoelic surface of the tube. As in the more proximal regions of the duct, some cilia arise within this tubular section. The ciliated ducts' opening into the hemocoel has no morphological specializations, although cilia originating from within and around the opening extend into the hemocoel.

The branches of the pore cell containing the ciliated ducts do not appear to be extending towards a particular point within the embryo. The distal tubular sections of the ducts and their openings were found in a variety of locations: just beneath nearby ectodermal cells, internally

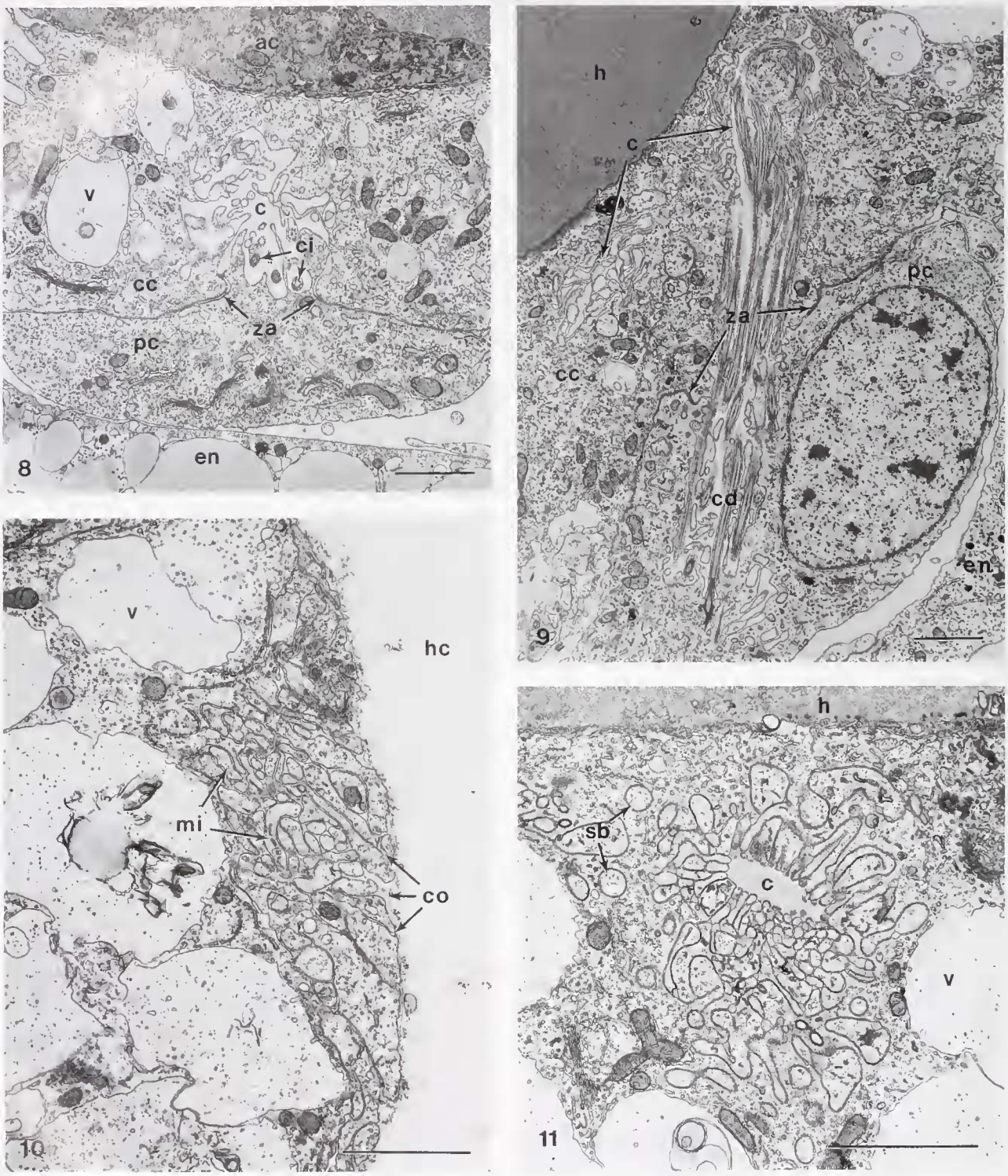


Figure 8. TEM showing the close association of the developing ciliated duct of the pore cell with the canaliculus of the crystal cell in an early trochophore of *Searlesia dira*

Figure 9. TEM of the same association seen in Figure 8, but in an older trochophore. Numerous cilia arising from the duct in the pore cell extend into the canaliculus of the crystal cell.

Figure 10. TEM of the opening of the crystal cell's canaliculus into the hemocoel in an early *S. dira* veliger. The opening of the canaliculus is no longer obstructed by the pore cell, but plications and microvilli from the canalicular wall reduce the opening to a web of anastomosing slits.

closer to the esophagus, or at some point between the esophagus and ectoderm.

In living early veligers, the ciliated ducts could be observed with Nomarski differential interference contrast microscopy. This stage was most suitable for observations because the larval kidneys were on the transparent neck region of the embryo. As seen in sections, the branching pattern and location of the pore cell relative to the crystal cell varied among embryos and between sides of the same embryo. Cilia within the ducts were seen actively beating, but those that extended for most of their length into the hemocoel beat irregularly and slowly. Occasionally the beat of the cilia would stop, at which time the ducts could not be discerned. In some preparations where the embryos were favorably oriented, two ciliated ducts within a single pore cell could be distinguished. However, only one could be discerned in most.

Uptake of fluorescein-labelled proteins by the absorptive cell. The initial endocytotic uptake and concentration of ferritin by absorptive cells raised questions regarding the kinds of material that normally would be removed from the capsular fluid by the these cells. If the larval kidneys were a storage site for wastes or foreign molecules picked up from the capsular fluid, then the absorptive cells may have reacted to the apoferritin protein coat as an exogenous molecule and removed it from the external milieu. To determine if the absorptive cells take up the endogenous albumen proteins to which they are normally exposed, embryos were soaked in a solution of FITC-labelled capsular albumen proteins for 10 min to 4 h, then rinsed with seawater. Under UV epi-illumination, the absorptive cells on these treated embryos fluoresced, whereas no other cells did at a visible level (Figs. 18, 19). This was true for all developmental stages examined, from early prefeeding trochophores with absorptive cells barely protruding from the embryo, to late veligers with absorptive cells that were diminishing in size. No cells in control embryos examined immediately upon removal from their capsules fluoresced.

Absorptive cells on embryos soaked in FITC-BSA fluoresced in a manner indistinguishable from those exposed to FITC-conjugated capsule albumen proteins. Therefore, FITC-BSA was used for many observations on *Searlesia dira* as well as other species. Some embryos, particularly trochophores during the nurse egg feeding stage of development, had swallowed enough of the FITC-BSA solution so that the gut lumen fluoresced. However, the level of this fluorescence was comparable to that of the soaking solution, which was low relative to the brilliance of the absorptive cells.

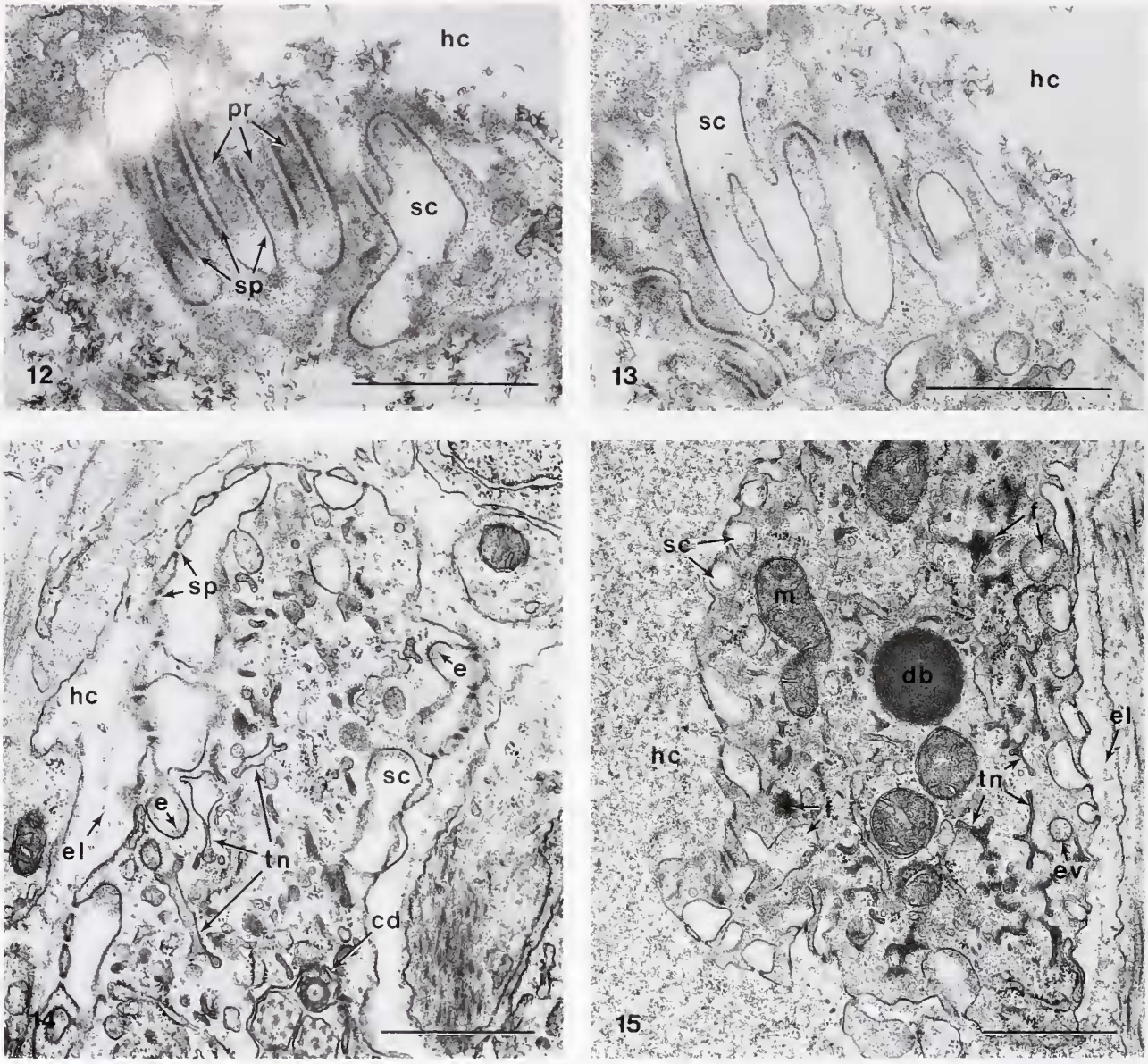
The absorptive cells did not take up unconjugated fluorescein in quantities visible even after soaking for 24 h. Furthermore, no fluorescence was seen in the absorptive cells when glucose, urea, BSA, capsular albumen, or ferritin was added to the fluorescein solution, even when the ferritin was taken up at levels detectable at the light level.

Contrary to the absorptive cells, the crystal cells did fluoresce in embryos that had been exposed to fluorescein solutions. In contrast to FITC-conjugated proteins, fluorescein entered the embryos so that the hemocoel fluoresced. However, the fluorescence of the crystal cell was greater than that of the hemocoel. Approximately 50% of the fluorescing crystal cells possessed an internal structure, possibly the canaliculus, whose fluorescence was markedly brighter than the rest of the cell. It was around 5 to 6.5 μm wide and 30 to 35 μm long, traversing about two-thirds of the cell. Although this is up to twice the diameter of the canaliculus visualized in sectioned material, no other structure in the cell more closely approximates these dimensions. The vacuoles containing the crystals were not distinguishable in these treated embryos under UV epi-illumination.

The pattern of fluorescence in the absorptive cells of the embryos exposed to FITC-BSA depended on the length of time of exposure and on the time since they were removed from the FITC-BSA. After 10 min of exposure, fluorescence could be detected in a few small heterophagosomes lying beneath the plasmalemma. With increasing time of exposure, fluorescence was seen in many more small heterophagosomes, in larger heterophagosomes further inside the cell and with greater intensity. The larger internal heterophagosomes were dimly fluorescent within 2 h, and brightly fluorescent after 4 h. After 7 h, the fluorescence in the larger heterophagosomes was uneven, suggesting a heterogeneity of protein concentrations within those structures. In embryos that were exposed to FITC-BSA for 3 h and then placed in normal seawater, few fluorescent small heterophagosomes were seen after another 3 h, with most of the fluorescence restricted to larger heterophagosomes. The fluorescence from the FITC-BSA taken in during the 3-h exposure was still present in the larger heterophagosomes three days later, the longest embryos survived after excapsulation. The fluorescence did not appear to diminish in intensity,

Figure 11. TEM of a cross section of a canaliculus in the pore cell of an *S. dira* early veliger. The lumen of the canaliculus is surrounded and often occluded by microvilli. There are numerous finger-like side branches of the canaliculus, each of which is filled by a microvillus.

Scale bars represent 2 μm; ac, absorptive cell; c, canaliculus; cc, crystal cell; cd, ciliated duct; ci, cilia; co, openings of ciliated duct; en, endoderm; h, heterophagosome of absorptive cell; hc, hemocoel; mi, microvilli; pc, pore cell; sb, side branches of the canaliculus each filled by a microvillus; v, vacuole; za, zonula adherens.



Figures 12 and 13. TEMs of two sequential grazing sections of the surface of the pore cell in *Searlesia dira*, illustrating how the interdigitations of finger-like processes form uniform 23 nm wide slit-pores and subsurface cisternae.

Figure 14. TEM of a pore cell in an *S. dira* early veliger, showing the extensive tubular network and endocytotic vesicles forming from the floor of the subsurface cisternae.

Figure 15. TEM of the pore cell of an *S. dira* early veliger whose hemocoel had been injected with ferritin 15 min prior to fixation. The ferritin solution still fills the hemocoel. Ferritin has entered the subsurface cisternae and was taken up in endocytotic vesicles which then fused with the tubular network.

Scale bars represent 1 μm; cd, ciliated duct; db, dark body; e, endocytotic invagination; el, external lamina; ev, endocytotic vesicle; f, ferritin; hc, hemocoel; m, mitochondrion; pr, cell processes; sc, subsurface cisternae; sp, slit-pore; tn, tubular network.

nor did it appear in other cells. However, within 15 h of exposure to FITC-BSA, the fluorescence was not clearly confined to the heterophagosomes; the cytoplasm of the absorptive cell also fluoresced, although dimly. This may indicate that labelled material is passed to the cytoplasm, that some heterophagosomes had burst, or that digestion of the FITC-BSA was releasing fluorescein which was diffusing into the cytoplasm.

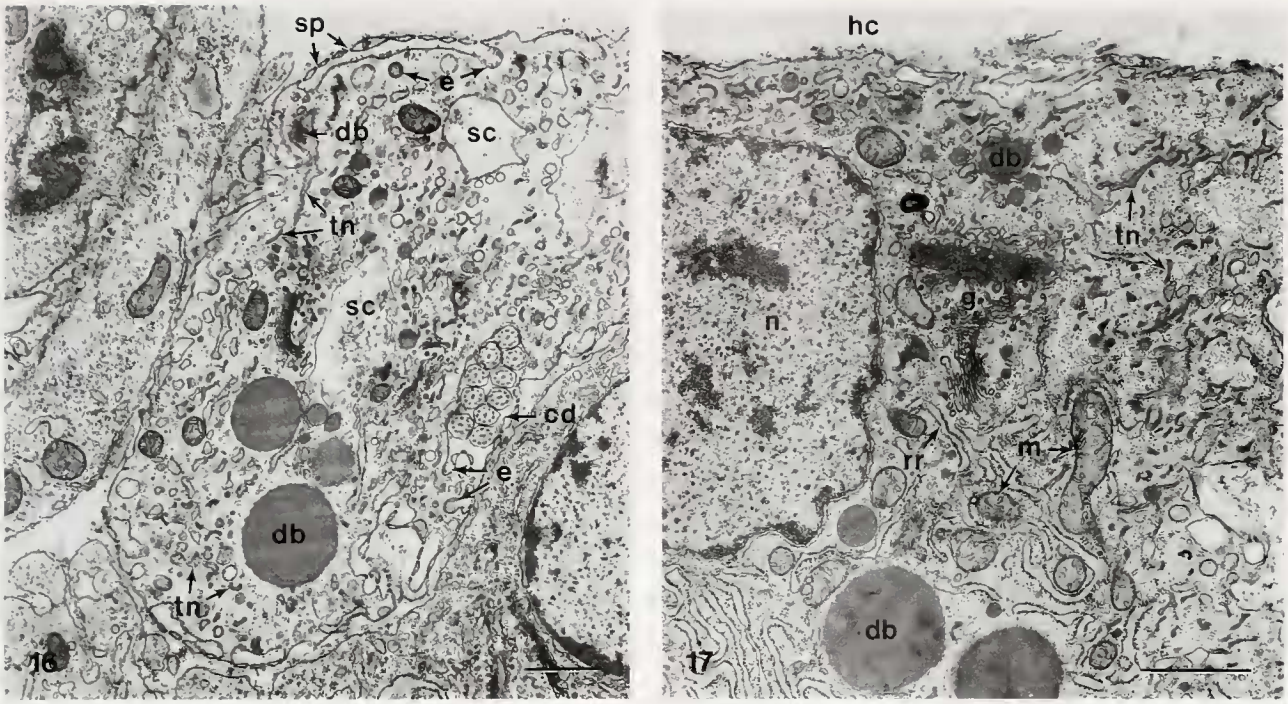


Figure 16. TEM showing the ciliated duct, endocytotic activity and dark bodies in the pore cell of a *Searlesia dira* early veliger. Note the early stage of dark body formation within the tubular network near the upper surface of the cell and the slit-pores within the ciliated duct.

Figure 17. TEM of the pore cell of an early trochophore of *S. dira* showing a large Golgi complex near a small dark body (near the upper cell surface) that is surrounded by smaller membrane-bound vesicles of similarly electron-dense material. Membranes are not always clearly seen around large dark bodies, as is evident at the bottom of this figure.

Scale bars represent 1 μm; cd, ciliated duct; db, dark body; e, endocytotic invaginations or vesicles; g, Golgi complex; hc, hemocoel; m, mitochondria; n, nucleus; rr, rough endoplasmic reticulum; sc, grazing sections of subsurface cisternae; sp, slit-pores; tn, tubular network.

Nucella canaliculata

As in all other species examined, the larval kidney complexes in *N. canaliculata* are located laterally in the neck region. They are first discernible in live embryos about the time the cephalic vesicle begins to enlarge on early trochophores. They increase rapidly in size and are prominent structures on late trochophores (Fig. 20). They reach their maximum size by the early veliger stage, a stage by which all nurse eggs have been eaten and the viscous capsular albumen has been absorbed or eaten.

The *N. canaliculata* absorptive cells become the largest cells in the embryo. They are larger than absorptive cells in any other species examined in this study except for those in *N. lamellosa*, which are comparable in size. They are hemispherical to almost spherical in shape. Their diameter can reach 300 μm, which means the two absorptive cells can comprise approximately 20% of the tissue volume of the embryo. The absorptive cells are fragile; their plasmalemmae are easily ruptured by handling of the embryos or by osmotic shock, slowly releasing intact heterophagosomes that may adhere to the outside of the cell (Fig. 21).

Only a general ultrastructural examination of the *N. canaliculata* larval kidney was completed because a satisfactory fixative was not found. Early trochophores through late veliger stages were sectioned for examination with light microscopy, but only the late trochophore stage was serially thin sectioned for TEM. The larval kidney in *N. canaliculata* consists of the same arrangement of an absorptive cell, a crystal cell and a pore cell as found in *S. dira*. The cytology of the absorptive cell in *N. canaliculata* is also similar. The external plasmalemma with its microvilli (Fig. 21) is endocytotically active. The heterophagosomes are heterogeneous in their density at both the light (Fig. 22) and TEM level. Rosettes of glycogen are present in the cytoplasm. Ferritin is rapidly taken in and stored in heterophagosomes, as is FITC-BSA (Fig. 23). These cells are endocytotically active throughout the intracapsular veliger phase of development. The crystal and pore cells differentiate in association with each

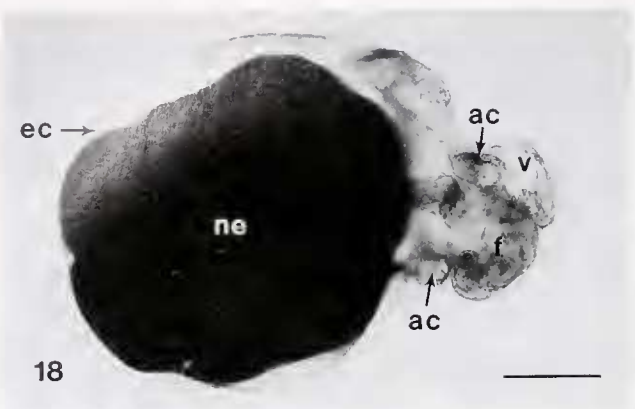


Figure 18. Light photomicrograph of a live *Searlesia dira* early veliger that had been soaked in a solution of FITC-capsular albumen for 3 h.

Figure 19. Epifluorescence photomicrograph of the same veliger seen in Figure 18, showing the bright absorptive cells that accumulated the fluorescein-labelled protein.

Scale bar represents 100 μm; ac, absorptive cell; ec, ectoderm; f, foot; ne, swallowed nurse eggs; v, velum.

other and develop the same ultrastructures they do in *S. dira*.

Presence of absorptive cells in different gastropod species. Embryos and some larvae from the three gastropod subclasses were tested for the presence of larval kidney absorptive cells. The species examined normally hatch either as veligers or postmetamorphic juveniles. Embryos were removed from their capsules, rinsed in seawater and placed in an FITC-BSA solution for at least 1 h before being examined under UV epi-illumination. Posthatching veligers of several species were obtained from cultures for testing.

In all seven prosobranch species tested that hatch as veligers, paired absorptive cells were found in prehatching stages (Table I). After hatching, veligers lost their absorptive cells within two or three days. Crystal cells were also detected in *Oenopota levidensis* veligers with polarized light. These cells were no longer detectable after the absorptive cells were resorbed during the second to third day after hatching.

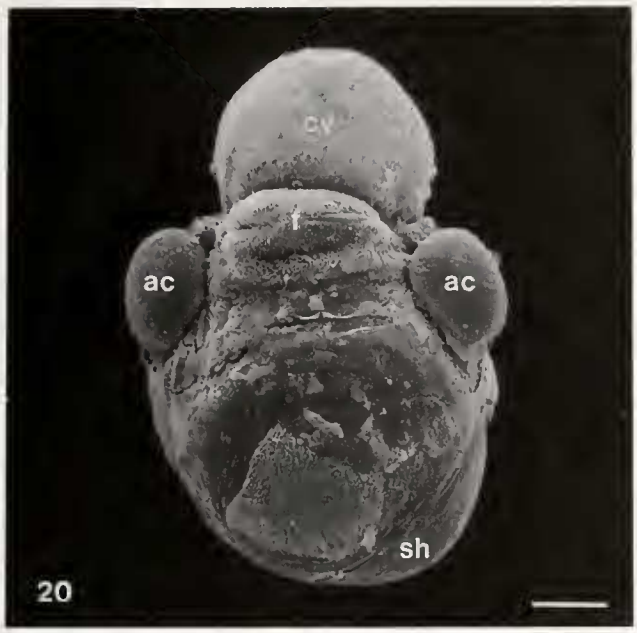
Of the twelve prosobranch species tested that hatch as juveniles, only ten had embryos possessing paired absorptive cells (Table I). In these species, the absorptive cells were resorbed or at least did not protrude from the embryos after intracapsular metamorphosis. *Littorina sitkana* and *Petaloconchus montereyensis* embryos were the only prosobranchs that did not show absorptive cells at some stage. The capsules of *L. sitkana* contain albumen, but those of *P. montereyensis* possess little or none as they are packed with nurse eggs. None of the opisthobranch or pulmonate embryos had absorptive cells.

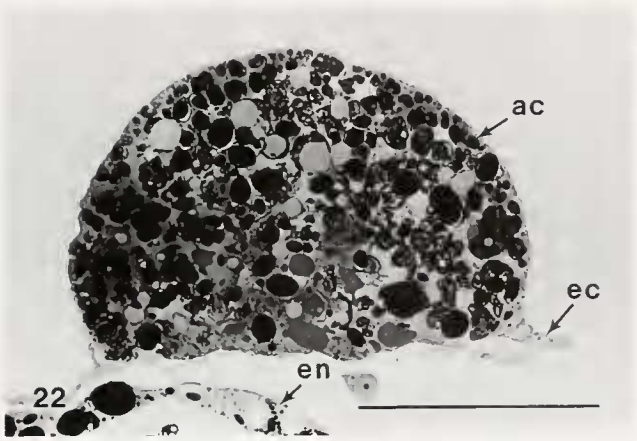
Discussion

Lack of evidence of waste accumulation

Marine prosobranchs are basically ammoniotelic, although uric acid may be found in various tissues (Nicol, 1960; Duerr, 1968). The larval kidneys, however, do not contain uric acid. The histochemical tests for urates in *Searlesia dira* and *Nucella canaliculata* embryos were negative, and there was no ultrastructural evidence for urates like that seen in terrestrial snails by Bouillon and Vandermeerssche (1962) and Pecheco (1971). The lack of uric acid and the positive PAS staining of the absorptive cells indicates that their large size is due to polysaccharides or polysaccharide-protein complexes and is unlikely due to accumulation of waste products.

Although no evidence for excretory activity by the larval kidneys was found during this study, previous reports indicating such a function may have been a consequence of several factors. First, these complexes of cells were called larval kidneys without any apparent definitive elucidation of their function, thus possibly biasing others as to their supposed role. Second, the delicate nature of the absorptive cells and the difficulty in fixing them well for ultrastructural studies help explain some of the observations of 'excretory' activity that have been reported. These large cells often break open, releasing intact heterophagosomes, before other structures visibly deteriorate in embryos removed from their capsules. Thus, the "excretory granules" seen by D'Asaro (1966, p. 895) emanating from the larval kidneys of *Thais haemastoma* and what Portmann (1930) thought were escaping waste-ladened wandering cells in *Buccinum undatum* embryos were likely to be heterophagosomes spilling from ruptured absorptive cells. The fragility of these cells may be due to the proteins they have accumulated causing osmotic shock when the embryos are removed from their capsules. This may be why these cells are difficult to fix well for ultrastructural study. The exocytosis cited as evidence for excretion by the absorptive cells in *Nucella lapillus* (Fioroni, 1985; Fioroni *et al.*, 1985) is similar to that seen in poorly fixed embryos





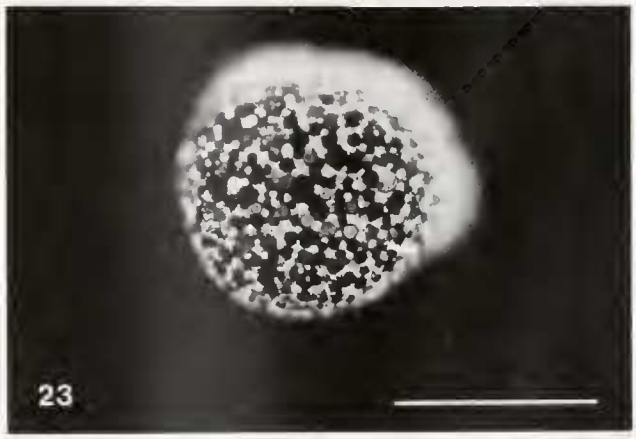


Figure 20. SEM of a *Nucella canaliculata* late trochophore showing the large size of the absorptive cells.

Figure 21. SEM of an absorptive cell on an early veliger of *N. canaliculata*. The cell has broken open revealing heterophagosomes. Microvilli are evident covering the external surface of the cell.

Figure 22. A light photomicrograph of a 1-μm thick section of an absorptive cell in an early veliger of *N. canaliculata*. Although the heterophagosomes are evident, the internal ultrastructure of the cell was greatly affected by fixation artifacts.

Figure 23. An epifluorescence photomicrograph of an absorptive cell on a live *N. canaliculata* early veliger that had been soaked for 1 h in a solution containing FITC-BSA. Heterophagosomes are clearly visible, with their level of fluorescence differing according to the amount of FITC-BSA they contain.

Scale bars represent 100 μm; ac, absorptive cell; cv, cephalic vesicle; ec, ectoderm; en, endoderm; f, foot; h, heterophagosome; sh, shell; v, velum.

of *S. dira* and *N. canaliculata*. It would seem illogical to describe the larval kidneys as specialized organs for encapsulated development that accumulate waste and then release it into the capsular fluid that bathes the embryos.

Absorptive cell

The absorptive cell is the only cell of the larval kidney complex that is exposed to the capsular fluid. It is clear from the experimental exposure of *S. dira* embryos to ferritin and FITC-capsular albumen that the absorptive cells rapidly take up protein from the external milieu by endocytosis and store it in heterophagosomes.

The early differentiation of the absorptive cells is consistent with the hypothesis that the albumen is an important source of nutrition for which capsulemates compete. The earlier an embryo has functional absorptive cells the

Table I

The presence of paired, laterally located fluorescent ectodermal absorptive cells in gastropod embryos or larvae exposed to FITC-BSA

Species (hatching stage*)	Fluorescing cells	Developmental stages tested
PROSOBRANCHIA		
Mesogastropoda		
Lacuna variegata (V)	+	Prehatching and 2-day-old veligers
Littorina sitkana (J)	–	Veligers
Petaloconchus montereyensis (J)	–	Before, during, and just after feeding on nurse eggs
Calyptraea fastigiata (V)	+	Late trochophore
Crepidula fornicata (V)	+ (weak)	Prehatching veligers
Crepidula adunca (J)	+	Early to late shell formation
Trichotropis cancellata (V)	+	Prehatching to 3-day-old veligers
Lamellaria sp. (V)	+	Prehatching veligers
Neogastropoda		
Nucella canaliculata (J)	+	Trochophores to late veligers
Nucella emarginata (J)	+	Trochophores, late veligers
Nucella lamellosa (J)	+	Trochophores to late veligers
Nucella lapillus (J)	+	Trochophores to early veligers
Nucella lima (J)	+	Trochophores to early veligers
Ocenebra japonica (J)	+	Early veligers
Ceratostoma foliatum (J)	+	Early trochophores
Amphissa columbiana (V)	+ (weak)	Prehatching veligers
	–	4 days post-hatching
Searlesia dira (J)	+	Trochophores to late veligers
Neptunea lyrata (J)	+	Late trochophores
Oenopota levidensis (V)	+	0–2-day-old veligers
	–	Veligers older than 2 days
OPISTHOBRANCHIA		
Onchidoris bilamellata (V)	–	1–2 days posthatching
Tritonia diomedea (V)	–	Prehatching veligers
PULMONATA		
Lymnaea stagnalis (J)	–	Before and during intracapsular albumen ingestion phase

* V = veliger; J = crawling juvenile.

greater are its chances of getting a larger share of the albumen in the capsule. The larval kidneys may be present as early as before (Franc, 1940) or just after (Eisawy and Sorial, 1974) the end of gastrulation. In living *S. dira* and *N. canaliculata* embryos, the absorptive cells are visible with light microscopy and are increasing in volume by the early trochophore stage. The cells reach their maximum size by the early veliger stage, a time by which no viscous albumen remains in the capsular fluid. There are great differences in absorptive cell sizes among prosobranch species, but this does not necessarily mean differences in the amount of albumen available. In species with smaller absorptive cells, the absorbed proteins may be passed to the rest of the embryo more rapidly or sooner than in species with larger absorptive cells.

It appears that when the larval kidneys of *S. dira* and *N. canaliculata* diminish in size they are totally resorbed, but the cytology of post-resorption stages has not been examined. No ultrastructural evidence of lysosomal activity was found in *S. dira* absorptive cells up through mid-veliger, the oldest stage examined. Resorption occurs during intracapsular metamorphosis in all the neogastropod species examined in this study that hatch as juvenile snails. However, Conklin (1897, p. 143) said the larval kidneys in *Crepidula fornicata* embryos "appear to be pinched off completely" and in *C. adunca*, Moritz (1939) said they were rolled off by the advancing mantle edge on the left side and by the larval heart on the right. In my examinations of these two species and on the confamilial *Calyptraea fastigiata*, the larval kidneys became almost spherical and then were easily dislodged when the capsules were opened. Observations through the clear walls of carefully handled capsules never revealed cast off absorptive cells. Rather, they were resorbed.

For species that hatch as veligers, the time of absorptive cell resorption varies. In *Thais savignyi* (Eisawy and Sorial, 1974) and *T. haemastoma* (Belisle and Byrd, 1980), they are resorbed before hatching. If the primary function of the absorptive cell is the uptake of capsular albumen during encapsulated development, then they are no longer needed after hatching. However, some species retain their absorptive cells for a few days after hatching. Tests with FITC-BSA indicate that the veligers of *Lacuna variegata*, *Trichotropis cancellata*, and *Oenopota levidensis* have

functional absorptive cells for at least two or three days after hatching. Similarly, the larval kidneys of *Trivia europea* persist after hatching, but only for a short time (Lebour, 1931). Thus, the absorptive cells appear to be principally an adaptation for intracapsular development.

The tests using FITC-BSA on a few opisthobranch and pulmonate species did not reveal structures that absorbed and concentrated proteins as did the prosobranch absorptive cells. Opisthobranch veligers may have an unpaired ectodermal larval or secondary kidney that consists of several types of cells near the anus (Bonar and Hadfield, 1974; Bickell and Chia, 1979). The veligers of *Aeolidia papillosa* are reported to possess protonephridia (Bartolomaeus, 1989). In pulmonates, some albumen is taken up generally during early embryogenesis by endocytosis, but most of the albumen is ingested after the gut becomes functional (Raven, 1946, 1975). Thus, prosobranch absorptive cells, as specialized embryonic ectodermal structures for the uptake of capsular albumen, may be unique among the invertebrate phyla.

Crystal cell

Although the function of the absorptive cell appears to be the uptake and storage of capsular albumen, the functions of the crystal and pore cells are less clear. The association of the crystal cell with most of the absorptive cell's internal surface suggests that the functions of these cells may be related, but evidence for this is presently lacking. The association may simply reveal an ancestral relationship.

In the crystal cell, vacuoles containing the crystals increase in number as the embryos develop. The crystals are likely to be made of a calcium salt. They are possibly calcium carbonate, for they were more soluble in acids than bases, were quickly dissolved in a solution of EGTA, which chelates Ca^{++} ions (Schmid and Reilley, 1957), and were stained by alizarin red S, which stains bone (Emmel and Cowdry, 1964) and calcium carbonate (Buddemeir and Kinzie, 1976). The crystals are in a readily soluble form, as indicated by their rapid dissolution after vacuolar membrane breakdown in non-salt-saturated solutions. The vacuolar membrane clearly protects the integrity of the crystals. The EGTA solutions did not affect the crystals in live embryos, but quickly dissolved those in heat-killed embryos. Although the crystals are more likely to contain calcium than uric acid, which dissolve more readily in high pH solutions (Thorpe, 1930), the function of sequestering calcium is unclear.

Endocytotic activity along the canaliculus in the crystal cell suggests that the contents of the vacuoles may originate from the canalicular lumen. However, the evidence indicates a very low level of endocytotic activity, and vacuoles are formed both before and after the separation of the pore cell and crystal cell when the canaliculus is first open to the hemocoel. Thus the relationship between the function of the canaliculus and crystal formation is uncertain. The canaliculi in the parietal cells of the vertebrate gastric mucosa are implicated in ion transport for HCl secretion (Ito and Winchester, 1963). However, the canaliculi in the crystal cells of *S. dira* are unlikely to be heavily involved in ion transport, for they lack the close association with numerous mitochondria that is characteristic in cells that do (Lawn, 1960; Copeland, 1967; Sardet *et al.*, 1979). Although the large surface area of the canaliculus due to the numerous microvilli and plications suggests a membrane-dependent function, the restricted nature of the lumen indicates an impeded movement of material either into or out of the canaliculus.

In experiments exposing *S. dira* embryos to solutions of fluorescein, the crystal cells fluoresced brightly. How the fluorescein is concentrated and what role the canaliculi play in the concentrating mechanism remains unknown.

Crystal cells have been reported in several other prosobranch embryos (Portmann, 1930; Franc, 1940; Fioroni, 1966, 1985). Franc (1940) described a vacuolated cell lying under the large external larval kidney cell in the embryos of *Oceanebra aciculata*. As in *S. dira*, the cell increased in size and became filled with vacuoles, but the vacuoles in *O. aciculata* became greenish in older developmental stages. In most of the species listed in Table I, the larval kidney cells had a yellowish color with the crystals in the crystal cells being a dark yellow to yellow-green. However, in additional observations on embryos of *Oceanebra* (either *O. interfossa* or *O. lurida*), the crystal cells were greenish-yellow with green granules (pers. obs.). There was no green color in young embryos, nor was there any green color elsewhere in the capsule. Therefore, the pigment likely was synthesized *de novo*.

Pore cell

Pore cells are a conspicuous cell type in the connective tissue of gastropods and bivalves (Sminia and Boer, 1973). There are also pore cells, referred to as nephrocytes or podocytes, in the blood spaces of decapod gill shafts (Wright, 1964; Strangways-Dixon and Smith, 1970; Foster and Howse, 1978; Taylor and Greenway, 1979). 'Nephrocyte' is an appropriate label for these cells if the material they remove from the hemolymph is an excretory product, but pore cells have also been implicated in the production of blood pigments in mollusks (Sminia and Boer, 1973; Skelding and Newell, 1975). Calling them 'pore cells' aptly describes their distinctive morphological features without implying function, whereas calling them 'podocytes' is misleading because of their morphological and functional differences with vertebrate kidney podocytes (Bloom and Fawcett, 1968).

In *S. dira* pore cells, material from the hemolymph passes through the slit-pores and is taken into the pore cell by endocytotic vesicles that form from the basal membranes of the subsurface cisternae. The external lamina may act as a filter, for in experimentally injected *S. dira* embryos the density of ferritin molecules inside the subsurface cisternae was markedly less than that in the hemocoel. Additional filtration is unlikely at the slit-pores, because their gaps are much wider than the diameter of the ferritin molecules. Furthermore, a membrane or diaphragm like that found in the nephrocytes of shrimp gills (Foster and Howse, 1978) was not seen spanning the slit-pores in *S. dira*. The material taken in by endocytosis is transferred to an extensive network of tubules, where it appears to be condensed to form large dark-staining bodies. A similar ultrastructure is found in pore cells in the gill blood vessels of a land crab, *Holthuisana transversa* (Taylor and Greenway, 1979). In general, pore cells appear to remove material from the hemolymph. However, the nature of this material and its fate remains to be elucidated.

A characteristic of the pore cells of *S. dira* and *N. canaliculata* that has not been found in pore cells in other species is the ciliated ducts. In *S. dira*, only the first of the two ciliated ducts develops in association with the canaliculus of the crystal cell, but eventually the two ducts are indistinguishable. They both originate near the cell center, contain a similar number of cilia, possess some slit-pores leading to the hemocoel somewhere along their length, and open into the hemocoel. The duct lumens in most places are only slightly wider than the cilia they contain. Movement of the cilia could move fluid down and out of the ducts. However, the low number of slit-pores to the hemocoel in the duct walls would result in a low flow rate. Indeed, no ferritin was found in ducts in injected embryos.

Comparison with protonephridia

It is logical to compare the 'larval kidneys' of *S. dira* and *N. canaliculata* to protonephridia because of their name, their bilateral location, and the fact that protonephridia have been described in other prosobranch, opisthobranch, and pulmonate gastropods, in chitons, and in bivalves (Erlanger, 1894; Brandenburg, 1966; Bartolomaeus, 1989; Ruthensteiner and Schaefer, 1991). This comparison is especially attractive in light of the pore cells' ciliated ducts with their slit-pore perforations to the hemocoel being so similar in morphology to many protonephridia. Although protonephridia are diverse in details of their morphology (Goodrich, 1945; Brandenburg, 1966, 1975; Wessing and Polenz, 1974; Wilson and Webster, 1974; Ruppert and Smith, 1988), they basically consist of a terminal cell or cells with one or more cilia within a cytoplasmic tube that leads to a channel cell or tubule that opens externally. The beating of the cilia draws hemolymph into the tubule through a weir of slits formed in some species by the interdigitation of microvilli from both the terminal and channel cells (Brandenburg, 1975; Kummel, 1975).

It is clear that the larval kidneys of *S. dira* and *N. canaliculata* are not functional protonephridia because a filtration weir never develops and the ciliated ducts lead not to the external milieu, but to the haemocoel. However, it is possible that the larval kidneys may have evolved from protonephridia and that the components have become disorganized and functionally altered. If this is so, the pore cell probably evolved from a protonephridial terminal cell. The pore cell's ciliated ducts are reminiscent of protonephridial 'flames,' and the slit-pores and subsurface cisternae are similar to that seen in a section of the terminal cell of the trochophore of *Pomatoceros triqueter* (Wessing and Polenz, 1974, Fig. 2b). The crystal and absorptive cells may have evolved from channel cells. The canaliculus may be the last remnant of the crystal cell's ancestral duct, with no such vestige remaining in the highly altered absorptive cell.

The presence of larval protonephridia may represent the ancestral condition in mollusks (Bartolomaeus, 1989), but gastropod embryos undergoing encapsulated development experience an environment different from the open-ocean surroundings experienced during development of their free-spawning, more primitive relatives. The egg capsules with their intracapsular fluid presumably create an osmotic environment different from that of seawater. This is supported by two observations made during this study. One is the fixation artifacts seen. These artifacts suggested an osmotic problem and were diminished when fixatives with osmolarities higher than seawater were used. The second is the bursting of the absorptive cells when embryos were carefully excapsulated into seawater. This was most noticeable in species where these cells are particularly large, and is presumably due to the osmotic imbalance between the concentrated proteins within the heterophagosomes and the seawater. Because protonephridia are reported to be involved in osmoregulation (Braun *et al.*, 1969) and the filtration and reabsorption of macromolecules from coelomic fluid (Smith and Ruppert, 1988), it would seem logical for encapsulation to reduce the selective pressures that maintain protonephridia as osmoregulatory organs. It is thus possible that the larval kidneys described herein may represent rudimentary protonephridia. The lack of organization among the components is not surprising if the need for functional protonephridia is lacking. The vestigial eyes of cave fish show a similar disorganization of components (Remane, 1971). The functions of the larval kidneys' cells also have changed. The pore and crystal cells have functions that

are presently unknown, and may simply be vestigial structures. The absorptive cell, however, has taken on a new role. It does not accumulate wastes as previously thought (*e.g.*, Franc, 1940), but is specialized for the acquisition of capsular albumen.

An ultrastructural study of the ontogeny of the larval kidney complex in *Nassarius reticulatus* might shed light on this question of homology, for Ruthensteiner and Schaefer (1991) found in their light microscopical study protonephridia with what appears to be an enlarged absorptive cell adjacent to the excretory pore. A similar study on the early ontogeny of the larval kidney complex of *S. dira* or *N. canaliculata* might help reveal the germ layer origins of the component cells and their homology with cells in other protonephridia. Investigators have described the larval kidneys as being ectodermal or also part mesodermal (Heymons, 1893; Conklin, 1897; Casteel, 1904; Pelseneer, 1911; D'Asaro, 1966). The absorptive cell in *S. dira* embryos is probably derived from an ectodermal cell for it has junctional complexes with the surrounding unspecialized ectodermal cells. However, the origins of the inner two cells is unclear. If they are protonephridial in nature, then they may be ectodermal (Goodrich, 1945). However, the pore cells in *S. dira* larval kidneys have similarities with pore cells in the connective tissues of other gastropods and bivalves (Sminia and Boer, 1973), which suggests that they may be mesodermal in origin. It should be noted here that protonephridial terminal cells (presumably ectodermal in origin) in the larvae of the polychaete *Sabellaria* transform into a podocyte (presumably a mesodermal cell) in the coelomic lining at metamorphosis (Smith and Ruppert, 1988), indicating that caution must be exercised in making assumptions about germ layer affinities (Ruppert and Smith, 1988).

Nutritional role of capsular albumen and other suggested functions

Although albumen in prosobranch egg capsules does not appear to be bactericidal (Rivest, 1981; Pechenik *et al.*, 1984), it may serve several other functions. It may be important physically by increasing the viscosity of the intracapsular fluid relative to seawater so that embryos can maneuver and feed on nurse eggs (Rivest, 1983). It may be important osmotically by protecting embryos from osmotic shock (Pechenik, 1983) or by aiding hatching (Hertling, 1928). The albumen, containing proteins and carbohydrates and sometimes lipid (Bayne, 1968; Stöckmann-Bosbach and Althoff, 1989), also can have nutritive value for prosobranch embryos. It can influence the developmental stage that hatches (Giglioli, 1955) or the size at hatching (Rasmussen, 1951; Rivest, 1986). Its nutritional value is suggested also by its endocytotic uptake in early cleavage stages (Elbers and Bluemink, 1960; Fioroni, 1977) and by the presence of a special transitory albumen digestive sac in the embryonic gut of some species (Portmann, 1955; Portmann and Sandmeier, 1965). In the closely allied pulmonates, protein gains in the growing embryo are positively correlated with protein losses in the capsular fluid (Morrill, 1964). Thus, it is apparent that regardless of its other functions, the proteinaceous albumen can be important nutritionally.

The albumen taken up by the absorptive cells in prosobranch larval kidneys is presumably used for embryonic nutrition, but the nutritional significance of this absorbed albumen is presently unknown. Assuming that the abortive cells' volume is due primarily to absorbed albumen proteins, the fact that these cells in *N. canaliculata* can comprise 20% of an embryo's tissue volume suggests that in this species the larval kidneys may procure a significant quantity of albumen. It would be difficult to empirically examine this in *Nucella* unless techniques for culturing encapsulated embryos improve. Although early embryos of *Conus pennaceus* can be reared to the veliger stage outside their capsules with no apparent detrimental effect (Perron, 1981), young *Nucella* embryos do not survive very long once removed from their capsule (Pechenik *et al.*, 1984; Stöckmann-Bosbach, 1988; pers. obs.).

Acknowledgments

This study benefitted from the advice of R. R. Strathmann, the late R. L. Fernald, the late C. G. Reed, C. Gabel, E. M. Eddy, and R. A. Cloney, who piqued my curiosity about prosobranch larval kidneys. E. Ruppert made numerous constructive criticisms on the manuscript, especially regarding the possible vestigial nature of the larval kidneys. R. Shimek provided *Oenopota levidensis* veligers, and R. Palmer provided *Nucella lima* egg capsules. The director of the Friday Harbor Laboratories kindly provided use of the laboratories' facilities. This study was supported in part by National Institutes of Health grants 5-T01-HD00266-10 and 1-T32-HD07183-01 and National Science Foundation grant OCE 7818608 (to R. R. Strathmann).

Literature Cited

Bartolomaeus, T. 1989. Larvale Nierenorgane bei *Lepidochiton cinereus* (Polyplacophora) und *Aeolidia papillosa* (Gastropoda). *Zoomorphology* **108:** 297–307.

Bayne, C. J. 1968. Histochemical studies on the egg capsules of eight gastropod molluscs. *Proc. Malacol. Soc. Lond.* **38:** 199–212.

Belisle, B. W., and W. Byrd. 1980. *In vitro* egg activation and maturation, and ultrastructural analysis of development in the marine prosobranch, *Thais haemastoma*. *Trans. Am. Microsc. Soc.* **99:** 111–127.

Bickell, L. R., and F. S. Chia. 1979. Organogenesis and histogenesis in the planktotrophic veliger of *Doridella steinbergae* (Opisthobranchia: Nudibranchia). *Mar. Biol.* **52:** 291–313.

Bloom, W., and D. W. Fawcett. 1968. *A Textbook of Histology, 9th ed.* W. B. Saunders Co., Philadelphia.

Bobretzky, N. 1877. Studien über die embryonal Entwicklung der Gastropoden. *Arch. Mikrobiol. Anat. Bd.* **13:** 95–169.

Bonar, D. B., and M. G. Hadfield. 1974. Metamorphosis of the marine gastropod *Phestilla sibogae* Bergh (Nudibranchia: Aeolidacea) I. Light and electron microscopic analysis of larval and metamorphic stages. *J. Exp. Mar. Biol. Ecol.* **16:** 227–255.

Bouillon, J., and G. Vandermeerssche. 1962. Electron microscope observations of the excretory organ of the pulmonary molluscs. *5th Int. Cong. Electron Microsc., Philadelphia* **2:** WW14–15.

Brandenburg, J. 1966. Die Reusenformem der Cyrtocyten. *Zool. Beit.* **12:** 346–417.

Brandenburg, J. 1975. The morphology of the protonephridia. *Fortsch. Zool.* **23:** 1–17.

Braun, G., G. Kümmel, and J. A. Mangos. 1966. Studies on the ultrastructure and function of a primitive excretory organ, the protonephridium of the rotifer *Asplanchna priodonta. Pflügers Arch.* **289:** 141–154.

Buddemeir, R. W., and R. A. Kinzie, III. 1976. Coral growth. *Oceanogr. Mar. Biol. Ann. Rev.* **14:** 183–225.

Casteel, D. B. 1904. The cell-lineage and early larval development of *Fiona marina,* a nudibranch mollusk. *Proc. Acad. Natl Sci. Phil. Ser. 3* **56:** 325–405.

Cavey, M., and R. Cloney. 1972. Fine structure and differentiation of ascidian muscle. I. Differentiated caudal musculature of *Distaplia occidentalis* tadpoles. *J. Morphol.* **138:** 349–374.

Conklin, E. G. 1897. The embryology of *Crepidula. J. Morphol.* **13:** 1–226.

Copeland, D. E. 1967. A study of salt secreting cells in the brine shrimp (*Artemia salina*). *Protoplasma* **63:** 363–384.

D'Asaro, C. N. 1966. The egg capsules, embryogenesis, and early organogenesis of a common oyster predator, *Thais haemastoma* (Gastropoda: Prosobranchia). *Bull. Mar. Sci.* **16:** 884–914.

Duerr, F. G. 1968. Excretion of ammonia and urea in seven species of marine prosobranch snails. *Comp. Biochem. Physiol.* **26:** 1051–1059.

Eisawy, A. M., and A. E. Sorial. 1974. Egg capsules and development of *Thais savignyi* Deshayes. *Bull. Inst. Oceanogr. Fish., Cairo* **4:** 237–258.

Elbers, P. F., and J. G. Bluemink. 1960. Pinocytosis in the developing egg of *Limnaea stagnalis* L. *Exp. Cell Res.* **21:** 619–622.

Emmel, V. M., and E. V. Cowdry. 1964. *Laboratory Technique in Biology and Medicine.* The Williams and Wilkens Co., Baltimore.

Erlanger, R. 1894. Zur Bildung des Mesoderms bei der *Paludina vivipara. Morph. Jb.* **22:** 113–118.

Fioroni, P. 1966. Zur Morphologie und Embryogenese des Darmtraktes und der transitorischen Organe bei Prosobranchiern (Mollusca, Gastropoda). *Rev. Suisse Zool.* **73:** 621–876.

Fioroni, P. 1977. On the peripheric resorption of albumen in gastropods and its significance for general embryology. *Zool. Jb. Anat. Bd* **98:** 181–202.

Fioroni, P. 1985. Struktur und Funktion der larvalen Zellen des Cephalopodiums bei jungen intrakapsulären Larven von *Nucella lapillus* (Gastropoda, Prosobranchia). *Zool. Beitr. N. F.* **29:** 103–117.

Fioroni, P., G. Sundermann, and D. P. Scheidegger. 1984. Die Ultrastruktur der freien Rhogocyten bei intrakapsulären Veligern von *Nucella lapillus* (Gastropoda, Prosobranchia, Stenoglossa). *Zool. Anz.* **212:** 193–202.

Fioroni, P., D. P. Scheidegger, and G. Sundermann. 1985. Die Ultrastruktur der Larvalnierne bei intrakapsulären Larven von *Nucella lapillus* (Gastropoda, Prosobranchia, Stenoglossa). *Zool. Jb. Anat.* **113:** 145–164.

Foster, C. A., and H. D. Howse. 1978. A morphological study on gills of the brown shrimp, *Penaeus aztecus. Tissue Cell.* **10:** 77–92.

Franc, A. 1940. Recherches sur le développement d'*Ocinebra aciculata* Lamark. *Bull. Biol. France Belgique* **74:** 327–345.

Franc, A. 1941. Sur les reins larvaires de certains Mollusques prosobranches. *C. R. Soc. Biol. Paris* **135:** 1487–1489.

Giglioli, M. E. 1955. The egg masses of the Naticidae (Gastropoda). *J. Fish. Res. Board Can.* **12:** 287–327.

Glaser, O. 1904. Excretory activities in the nuclei of gastropod embryos. *Am. Nat.* **38:** 513–516.

Goodrich, E. S. 1945. The study of nephridia and genital ducts since 1895. *O. J. Microsc. Sci.* **86:** 113–392.

Hertling, H. 1928. Beobactungen und Versuche an den Eiern von *Littorina* und *Lacuna.* Bedeutung der Eihüllen. Entwicklung im natürlichen und abgeänderten Medium. *Wiss. Meers. Abt. Helgoland* **17:** 1–49.

Heymons, R. 1893. Zur Entwicklungsgeschicte von *Umbrella mediterranea,* Lam. *Z. Wiss. Zool.* **56:** 245–298.

Humason, G. L. 1972. *Animal Tissue Technique, 3rd ed.* W. H. Freeman and Co., San Francisco.

Ito, S., and R. J. Winchester. 1963. The fine structure of the gastric mucosa in the bat. *J. Cell Biol.* **16:** 541–577.

Kummel, G. 1975. The physiology of protonephridia. *Fortsch. Zool.* **23:** 18–32.

Lawn, A. M. 1960. Observations on the fine structure of the gastric parietal cells of the rat. *J. Biophys. Biochem. Cytol.* **7:** 161–166.

Lebour, M. 1931. The larval stages of *Trivia europea. J. Mar. Biol. Assoc. U. K.* **17:** 819–831.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:** 409–414.

Moritz, C. E. 1939. Organogenesis in the gasteropod *Crepidula adunca* Sowerby. *Univ. Calf. Publ. Zool.* **43:** 217–248.

Morrill, J. B. 1964. Protein content and dipeptidase activity of normal and cobalt-treated embryos of *Lymnaea palustris. Acta Embryol. Morphol. Exp.* **7:** 131–142.

Nicol, J. A. C. 1960. *The Biology of Marine Animals.* Interscience Publishers, New York.

Pearse, A. G. E. 1972. *Histochemistry, Vol. II, 3rd ed.* The Williams and Wilkens Co., Baltimore.

Pechecn, J. 1971. Fine structure of concretions in the kidney epithelium of *Pomacea urceus* (Gastropoda: Prosobranchia). *Acta Biol. Venez.* **7:** 443–458.

Pechenik, J. 1983. Egg capsules of *Nucella lapillus* (L.) protect against low-salinity stress. *J. Exp. Mar. Biol. Ecol.* **71:** 165–179.

Pechenik, J. A., S. C. Chang, and A. Lord. 1984. Encapsulated development of the marine prosobranch gastropod *Nucella lapillus. Mar. Biol.* **78:** 223–229.

Pelseneer, R. 1911. Recherches sur l'embryologie des gastropodes. *Acad. R. Belg. Mem. Cl. Sci. Collect.* **3:** 1–167.

Perron, F. E. 1981. Larval growth and metamorphosis of *Conus* (Gastropoda: Toxoglossa). *Pac. Sci.* **35:** 25–38.

Portmann, A. 1930. Die Larvenniern von *Buccinum undatum* L. *Z. Zell. Mikro. Anat.* **10:** 401–410.

Portmann, A. 1955. La métamorphose "abritée" de *Fusus* (Gast. Prosobranches). *Rev. Suisse Zool.* **62:** 236–252.

Portmann, A., and E. Sandmeier. 1965. Die Entwicklung von Vorderdarm, Macromeren und Enddarm unter dem Einfluss von Nähreiern bei *Buccinum, Murex* und *Nucella* (Gastrop. Prosobranchia). *Rev. Suisse Zool.* **72:** 187–204.

Rasmussen, E. 1951. Faunistic and biological notes on marine invertebrates II. The eggs and larvae of some Danish Marine Gastropods. *Vidensk. Medd Dan. Naturhist. Foren.* **113:** 201–249.

Raven, C. P. 1946. The development of the egg of *Lymnaea stagnalis* L. from the first cleavage till the trochophore stage, with special reference to its "chemical embryology." *Arch. Neerl. Zool.* **7:** 353–434.

Raven, C. P. 1975. Development. Pp. 367–400 in *Pulmonates*, V. Fretter and J. Peake, eds. Academic Press, New York.

Remane, A. 1971. *Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik.* Otto Koeltz, Koenigstein-Taunus (reprint of the 1956 ed. published by Geest & Portig, Leipzig).

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17:** 208–211.

Richardson, K. C., L. Jarrett, and E. H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* **35:** 313–323.

Rivest, B. R. 1981. Nurse egg consumption and the uptake of albumen in the embryonic nutrition of marine snails. Ph.D. dissertation. University of Washington, Seattle.

Rivest, B. R. 1983. Development and the influence of nurse egg allotment on hatching size in *Searlesia dira* (Reeve, 1846) (Prosobranchia: Buccinidae). *J. Exp. Mar. Biol. Ecol.* **69:** 217–241.

Rivest, B. R. 1986. Extra-embryonic nutrition in the prosobranch gastropod *Urosalpinx cinerea* (Say, 1822). *Bull. Mar. Sci.* **39(2):** 498–505.

Ruppert, E. E., and P. R. Smith. 1988. The functional organization of filtration nephridia. *Biol. Rev.* **63:** 231–258.

Ruthensteiner, B., and K. Schaefer. 1991. On the protonephridia and 'larval kidneys' of *Nassarius* (*Hinia*) *reticulatus* (Linnaeus) (Caenogastropoda). *J. Moll. Stud.* **57:** 323–329.

Sardet, C., M. Pisam, and J. Maetz. 1979. The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. *J. Cell Biol.* **80:** 96–117.

Schmid, R. W., and C. N. Reilley. 1957. New complexon for titration of calcium in the presence of magnesium. *Anal. Chem.* **29:** 264–268.

Skelding, J. M., and P. F. Newell. 1975. On the functions of the pore cells in the connective tissue of terrestrial pulmonate molluscs. *Cell Tissue Res.* **156:** 381–390.

Sminia, T., and H. H. Boer. 1973. Haemocyanin production in pore cells of the snail *Lymnaea stagnalis*. *Z. Zellforsch.* **145:** 443–445.

Smith, P. R., and E. E. Ruppert. 1988. Nephridia. In *Ultrastructure of the Polychaeta* W. Westheide and C. O. Hermans, eds. *Microfauna Marina* **4:** 231–262.

Stöckmann-Bosbach, R. 1988. Early stages of the encapsulated development of *Nucella lapillus* (Linnaeus) (Gastropoda, Muricidae). *J. Moll. Stud.* **54:** 181–196.

Stöckmann-Bosbach, R., and J. Althoff. 1989. A correlated morphological and biochemical study of capsular fluid of *Nucella lapillus* (Gastropoda: Prosobranchia: Muricidae). *Mar. Biol.* **102:** 283–289.

Strangways-Dixon, J., and D. S. Smith. 1970. The fine structure of gill 'podocytes' in *Panulirus argus* (Crustacea). *Tissue Cell* **2:** 611–624.

Taylor, H. H., and P. Greenway. 1979. The structure of the gills and lungs of the arid-zone crab, *Holthuisana* (*Austrothelphusa*) *transversa* (Brachyura: Sundathelphusidae) including observations on arterial vessels within the gills. *J. Zool., Lond.* **189:** 359–384.

Thorpe, E. 1930. *A Dictionary of Applied Chemistry.* Longmans, Green and Co., London.

Tomlinson, S. G. 1987. Intermediate stages in the embryonic development of the gastropod *Ilyanassa obsoleta*: a scanning electron microscope study. *Int. J. Invert. Reprod. Dev.* **12:** 253–280.

Wessing, A., and A. Polenz. 1974. Structure, development and function of the protonephridia in trochophores of *Pomatoceros triqueter* (Annelida, Polychaeta, Sedentaria). *Cell Tissue Res.* **156:** 21–33.

Wilson, R. A., and L. A. Webster. 1974. Protonephridia. *Biol. Rev.* **49:** 127–160.

Wood, R. L., and J. H. Luft. 1965. The influence of buffer systems on fixation with osmium tetroxide. *J. Ultrastruct. Res.* **12:** 22–45.

Wright, K. A. 1964. The fine structure of the nephrocyte of the gills of two marine decapods. *J. Ultrastruct. Res.* **10:** 1–13.